

WHAT IS CLAIMED IS:

1. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

- 5 (a) preparing a probe A and a probe B,
 said probe A being a first probe which has a
 sequence F' complementary to a first partial sequence F
 of the target nucleic acid and a binding molecule bound
 to the sequence F', and
10 said probe B being a second probe which has a
 sequence S' complementary to a second partial sequence
 S of the target nucleic acid and a flag bound to the
 sequence S', where said flag is a double-stranded
 sequence and has a marker substance in one of the
15 double strand;
- (b) hybridizing the first probe A with the first
 partial sequence F of the target nucleic acid and
 hybridizing the second probe B with the second partial
 sequence S of the target nucleic acid;
- 20 (c) ligating the first probe A and the second
 probe B both being hybridized with the target nucleic
 acid, thereby obtaining a probe (A+B);
- (d) binding the binding molecule to a substance
 capable of being paired up therewith, thereby
25 recovering the probe (A+B); and
- (e) recovering a single-stranded nucleic acid
 having the marker substance of the double stranded

nucleic acid constituting the flag and detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid in the specimen.

2. A method of detecting or quantifying target
5 nucleic acids N1-Nn (n is an integer of 2 or more),
each having a predetermined sequence, in a specimen,
comprising:

(a) preparing probes A1-An (n is an integer of 2
or more) and probes B1-Bn (n is an integer of 2 or
10 more),

said probes A1-An being first probes which
respectively have sequences F1'-Fn' (n is an integer of
2 or more) complementary to first partial sequences
F1-Fn (n is an integer of 2 or more) of the target
15 nucleic acids and a binding molecule bound to each of
the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more)
being second probes which respectively have sequences
S1'-Sn' (n is an integer of 2 or more) complementary to
20 second partial sequences S1-Sn (n is an integer of 2 or
more) of the target nucleic acids and flags bound to
the sequences S1'-Sn', where each of said flags is a
double-stranded sequence and has a marker substance in
one of the double strand; and

25 (b) respectively hybridizing the first probes
A1-An with the first partial sequences F1-Fn of the
target nucleic acids, and simultaneously hybridizing

the second probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids, respectively;

5 (c) respectively ligating the first probes A1-An and the second probes B1-Bn, both being hybridized with the target nucleic acids, respectively, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

10 (d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probes (A1+B1)-(An+Bn); and

15 (e) recovering a single-stranded nucleic acid having the marker substance from the double-stranded nucleic acid constituting each of the flags and detecting or quantifying the marker substance, thereby detecting or quantifying each of the target nucleic acids N1-Nn in the specimen.

20 3. A method of detecting or quantifying a target nucleic acid having a predetermined sequence, in a specimen, comprising:

(a) preparing a probe A and a probe B,
said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a tag sequence Tg bound to the sequence F', and
25 said probe B being a second probe which has a sequence S' complementary to a second partial sequence

S of the target nucleic acid and a marker substance bound to the sequence S'

5 (b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second partial sequence S of the target nucleic acid;

10 (c) ligating the probe A and the probe B, both being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) dissociating the probe (A+B) from the target nucleic acid;

15 (e) hybridizing the tag sequence Tg with a sequence Tg' complementary to the tag sequence Tg, thereby recovering the probe (A+B); and

(f) detecting or quantifying the marker substance in the probe (A+B) recovered, thereby detecting or quantifying the target nucleic acid in the specimen.

20 4. A method of detecting or quantifying nucleic acids N1-Nn, each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

25 said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences

F1-Fn (n is an integer of 2 or more) of the target nucleic acids N1-Nn (n is an integer of 2 or more) and tag sequences Tg1-Tgn bound to the sequences F1'-Fn', and

5 said probes B1-Bn being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target nucleic acids N1-Nn and the marker substance bound to
10 each of the sequences S1'-Sn' of the target nucleic acid;

 (b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of
15 the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

 (c) respectively ligating probes A1-An and the
20 probes B1-Bn, both being hybridized with the target nucleic acids, respectively, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

 (d) dissociating the probes (A1+B1)-(An+Bn) from the target nucleic acids;

25 (e) hybridizing sequences Tg1-Tgn respectively with sequences Tg1'-Tgn' complementary to the tag sequences Tg1-Tgn, thereby recovering the probes

(A1+B1)-(An+Bn); and

(f) detecting or quantifying the marker substance
in the probes (A1+B1)-(An+Bn) recovered, thereby
detecting or quantifying the target nucleic acids N1-Nn
in the specimen.

5 5. A method of detecting or quantifying a target
nucleic acid having a predetermined sequence in a
specimen, comprising:

(a) preparing a probe A and a probe B,

10 said probe A being a first probe which has a
sequence F' complementary to a first partial sequence F
of the target nucleic acid and a tag sequence Tg bound
to the sequence F', and

15 said probe B being a second probe which has a
sequence S' complementary to a second partial sequence
S of the target nucleic acid, a flag sequence FL bound
to the sequence S', and a marker substance bound to the
flag sequence FL;

20 (b) mixing the probe A, the probe B, and the
specimen, thereby hybridizing the probe A with the
first partial sequence F of the target nucleic acid and
simultaneously hybridizing the probe B with the second
partial sequence S of the target nucleic acid;

25 (c) ligating the probe A and the probe B, both
being hybridized with the target nucleic acid, thereby
obtaining a probe (A+B);

(d) dissociating the probe (A+B) from the target

nucleic acid;

(e) hybridizing the tag sequence Tg contained in the probe (A+B) with a sequence Tg' complementary to the tag sequence Tg, thereby dissociating the probe
5 (A+B); and

(f) recovering a portion containing at least the probe B from the probe (A+B) hybridized with the sequence Tg';

(g) hybridizing the flag sequence FL recovered
10 with a nucleic acid sequence FL' complementary to the flag sequence FL, thereby specifically recovering the portion containing at least probe B; and

(h) selectively detecting the marker substance contained in the portion containing at least the probe
15 B recovered, thereby detecting or quantifying the target nucleic acid in the specimen.

6. A method of detecting or quantifying nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

20 (a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of
25 2 or more) complementary to first partial sequences F1-Fn of the target nucleic acids N1-Nn (n is an integer of 2 or more), respectively, and tag sequences

Tg1-Tgn bound to the sequences F1'-Fn', respectively,
and

said probes B1-Bn being second probes which
respectively have sequences S1'-Sn' (n is an integer of
2 or more) complementary to second partial sequences
S1-Sn (n is an integer of 2 or more) of the target
nucleic acids N1-Nn, flag sequences FL1-FLn bound to
the sequences S1'-Sn', and a marker substance bound to
each of the flag sequences FL1'-FLn';

10 (b) mixing the probes A1-An, the probes B1-Bn,
and the specimen, hybridizing probes A1-An respectively
with the first partial sequences F1-Fn of the target
nucleic acids N1-Nn, and simultaneously hybridizing the
probes B1-Bn with the second partial sequences S1-Sn of
15 the target nucleic acids N1-Nn, respectively;

(c) respectively ligating probes A1-An and second
probes B1-Bn, both being hybridized with the target
nucleic acids, thereby obtaining probes (A1+B1)-(An+Bn)
(n is an integer of 2 or more);

20 (d) dissociating the probes (A1+B1)-(An+Bn) from
the target nucleic acids;

(e) hybridizing tag sequences Tg1-Tgn contained
in the probes (A1+B1)-(An+Bn) with sequences Tg1'-Tgn'
complementary to the tag sequences Tg1-Tgn, thereby
25 dissociating the probes (A1+B1)-(An+Bn); and

(f) recovering portions respectively containing
at least the probes B1-Bn, from the probes

(A1+B1)-(An+Bn) hybridized with the sequence Tg1'-Tgn';

(g) hybridizing the flag sequences FL1-FLn with nucleic acid sequences FL1'-FLn' complementary to the flag sequences FL1-FLn, thereby specifically recovering the portions respectively containing at least probes B1-Bn; and

(h) selectively detecting the marker substance contained in the portions respectively containing at least the probes B1-Bn recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

7. A method of detecting or quantifying nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn of the target nucleic acids N1-Nn (n is an integer of 2 or more) and tag sequences Tg1-Tgn bound to the sequences F1'-Fn', respectively, and

said probes B1-Bn being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or more) of the target

nucleic acids N1-Nn, and a marker substance bound to each of the sequences S1'-Sn'

5 (b) mixing the probes A1-An, the probes B1-Bn, and the specimen, thereby hybridizing probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn, and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

10 (c) respectively ligating probes A1-An and second probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-(An+Bn) (n is an integer of 2 or more);

15 (d) hybridizing tag sequences Tg1-Tgn with sequences Tg1'-Tgn' complementary to the tag sequences Tg1-Tgn, thereby recovering the probes (A1+B1)-(An+Bn); and

20 (e) detecting or quantifying the marker substance contained in the probes (A1+B1)-(An+Bn) recovered, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen,

wherein Tm values of the tag sequences Tg1-Tgn are higher than Tm values of sequences F1-Fn and sequences S1-Sn.

25 8. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

(a) preparing a probe A and a probe B,
said probe A being a first probe which has a
sequence F' complementary to a first partial sequence F
of the target nucleic acid and a binding molecule bound
5 to the sequence F', and

said probe B being a second probe which has a
sequence S' complementary to a second partial sequence
S of the target nucleic acid and a flag sequence FL
consisting of 4 units bound to the sequence S', where
10 said flag FL sequence hybridizes with a sequence FL'
bound to the sequence S' to form a double-stranded
sequence; and

(b) mixing the probe A, probe B and the specimen,
thereby hybridizing the probe A with the first partial
15 sequence F of the target nucleic acid, and
simultaneously hybridizing the second probe B with the
second partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both
being hybridized with the target nucleic acid, thereby
20 obtaining a probe (A+B);

(d) binding the binding molecule to a substance
capable of being paired up therewith, thereby
recovering the probe (A+B); and

(e) denaturing the double-stranded flag sequence
25 of the probes (A+B) recovered into single-stranded flag
sequence;

(f) hybridizing the single-stranded flag sequence

with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the primers to form a complementary strand of the flag sequence FL, thereby obtaining a double strand;

(g) binding a binding molecule B with a substance capable of being paired with the binding molecule B, thereby recovering the double strand; and

(h) detecting or quantifying the target substance L, thereby detecting or quantifying the target nucleic acid in the specimen.

9. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to

second partial sequences S_1-S_n (n is an integer of 2 or more) of the target nucleic acids, and flag sequences FL_1-FL_n each consisting of 4 units, bound to the sequences $S_1'-S_n'$, where said flag sequences FL_1-FL_n hybridize respectively with sequences $FL_1'-FL_n'$ bound to the sequences $S_1'-S_n'$ to form double-stranded sequences; and

(b) mixing the probes A_1-A_n , the probes B_1-B_n , and the specimen, thereby hybridizing probes A_1-A_n respectively with the first partial sequences F_1-F_n of the target nucleic acids N_1-N_n , and simultaneously hybridizing the probes B_1-B_n with the second partial sequences S_1-S_n of the target nucleic acids N_1-N_n ;

(c) respectively ligating the probes A_1-A_n and the probes B_1-B_n , both being hybridized with the target nucleic acids N_1-N_n , thereby obtaining probes $(A_1+B_1)-(A_n+B_n)$;

(d) binding each of the binding molecules to a substance capable of being paired up therewith, thereby recovering the probes $(A_1+B_1)-(A_n+B_n)$; and

(e) denaturing double-stranded flag sequences of the probes $(A+B)-(A_n+B_n)$ recovered into single-stranded flag sequences;

(f) hybridizing the single-stranded flag sequences FL_1-FL_n with two primers one of which has a binding molecule B and the other of which has a marker substance L, and extending the two primers, to form

complementary strands of the flag sequences FL1-FLn,
thereby obtaining double strands;

(g) binding a binding molecule B with a substance
capable of being paired therewith, thereby recovering
5 the double strands; and

(h) detecting or quantifying the marker substance
L, thereby detecting or quantifying the target nucleic
acids N1-Nn in the specimen.

10 10. A method of detecting or quantifying a target
nucleic acid having a predetermined sequence in a
specimen comprising:

(a) preparing a probe A and a probe B,
said probe A being a first probe which has a
sequence F' complementary to a first partial sequence F
15 of the target nucleic acid and a binding molecule bound
to the sequence F', and

said probe B being a second probe which has a
sequence S' complementary to a second partial sequence
S of the target nucleic acid and a flag consisting of 4
20 units bound to the sequence S', where said flag FL is a
double-stranded sequence; and

(b) mixing the probe A, the probe B, and the
specimen, thereby hybridizing the probe A with the
first partial sequence F of the target nucleic acid and
25 simultaneously hybridizing the probe B with the second
partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B, both

being hybridized with the target nucleic acid, thereby obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby
5 recovering the probe (A+B); and

(e) denaturing the double-stranded nucleic acid constituting the flag into single-stranded nucleic acid;

(f) amplifying the single-stranded nucleic acid
10 present in a liquid phase by PCR, thereby performing an encode reaction;

(g) performing transcription of a sequence FL' complementary to the single stranded flag sequence obtained by the encode reaction, by use of two primers
15 one of which is a primer having another binding molecule and the other of which is a primer having a marker substance, thereby performing a decode reaction;

(h) binding said another binding molecule to a substance being paired up therewith, recovering a
20 nucleic acid molecule obtained by the decode reaction; and

(i) detecting or quantifying the marker substance, thereby detecting or quantifying the target nucleic acid.

25 11. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more), each having a predetermined sequence, in a specimen,

comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

5 said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of the
10 sequences F1'-Fn', and

 said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences S1'-Sn' (n is an integer of 2 or more) complementary to second partial sequences S1-Sn (n is an integer of 2 or
15 more) of the target nucleic acids and flag sequences FL1-FLn each consisting of 4 units, bound to the sequences S1'-Sn';

(b) mixing the first probes A1-An, the second probes B1-Bn, and the specimen, thereby hybridizing the
20 probes A1-An respectively with the first partial sequences F1-Fn of the target nucleic acids N1-Nn and simultaneously hybridizing the probes B1-Bn with the second partial sequences S1-Sn of the target nucleic acids N1-Nn, respectively;

25 (c) respectively ligating the probes A1-An and the probes B1-Bn, both being hybridized with the target nucleic acids N1-Nn, thereby obtaining probes

(A1+B1)-(An+Bn) (n is an integer of 2 or more);

(d) binding the binding molecule to a substance capable of being paired up therewith, to recover the probes (A1+B1)-(An+Bn), and thereafter performing an
5 encode reaction of each of the flags FL1-FLn; and

(e) performing a decode reaction of the sequences FL1'-FLn' complementary to the flags FL1-FLn obtained by the encode reaction; and

(f) detecting or quantifying the nucleic acid
10 molecules obtained by the decode reaction, thereby detecting or quantifying the target nucleic acids N1-Nn in the specimen.

12. A method of detecting or quantifying target nucleic acids N1-Nn (n is an integer of 2 or more),
15 each having a predetermined sequence, in a specimen, comprising:

(a) preparing probes A1-An (n is an integer of 2 or more) and probes B1-Bn (n is an integer of 2 or more),

20 said probes A1-An being first probes which respectively have sequences F1'-Fn' (n is an integer of 2 or more) complementary to first partial sequences F1-Fn (n is an integer of 2 or more) of the target nucleic acids and a binding molecule bound to each of
25 the sequences F1'-Fn', and

said probes B1-Bn (n is an integer of 2 or more) being second probes which respectively have sequences

S1'-Sn' (n is an integer of 2 or more) complementary to
second partial sequences S1-Sn (n is an integer of 2 or
more) of the target nucleic acids and flag sequences
FL1-FLn each consisting of 4 units, bound to the
5 sequences S1'-Sn', respectively,

(b) mixing the probes A1-An, the probes B1-Bn,
and the specimen, thereby hybridizing probes A1-An
respectively with the first partial sequences F1-Fn of
the target nucleic acids N1-Nn, and simultaneously
10 hybridizing the probes B1-Bn with the second partial
sequences S1-Sn of the target nucleic acids N1-Nn,
respectively;

(c) respectively ligating the probes A1-An and
the probes B1-Bn, both being hybridized with the target
15 nucleic acids N1-Nn, thereby obtaining probes (A1+B1)-
(An+Bn);

(d) binding each of the binding molecules to a
substance capable of being paired up therewith to
recover the probes (A1+B1)-(An+Bn), and thereafter
20 performing an encode reaction for each of the flags
FL1-FLn; and

(e) performing a decode reaction of the sequences
F11'-FLn' complementary to the flags FL1-FLn (n is an
integer of 2 or more) obtained by the encode reaction;
25 and

(h) detecting the nucleic acid molecules
obtained by the decode reaction, thereby detecting or

quantifying the target nucleic acids N1-Nn in the specimen,

wherein 2 units of 4 units are sequences functioning as primers for PCR amplification.

5 13. A method of detecting or quantifying a target nucleic acid having a predetermined sequence in a specimen comprising:

(a) preparing a probe A and a probe B,

10 said probe A being a first probe which has a sequence F' complementary to a first partial sequence F of the target nucleic acid and a binding molecule bound to the sequence F', and

15 said probe B being a second probe which has a sequence S' complementary to a second partial sequence S of the target nucleic acid and a flag consisting of 4 units bound to the sequence S', where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bounded to each other sequentially in the order
20 mentioned; and

(b) mixing the probe A, the probe B, and the specimen, thereby hybridizing the probe A with the first partial sequence F of the target nucleic acid and simultaneously hybridizing the probe B with the second
25 partial sequence S of the target nucleic acid;

(c) ligating the probe A and the probe B both being hybridized with the target nucleic acid, thereby

obtaining a probe (A+B);

(d) binding the binding molecule to a substance capable of being paired up therewith, thereby recovering the probe (A+B); and

5 (e) denaturing the double-stranded nucleic acid constituting the flag into a single-stranded nucleic acid;

(f) hybridizing the single-stranded nucleic acid obtained in a liquid phase with sequences complementary to sequences D11-D1n labeled with a marker substance,
10 as primers,

(g) extending the primers hybridized

(h) denaturing a double-stranded nucleic acid having primers into a single-stranded nucleic acid;

15 (i) hybridizing the sequences D01-D0n specifically with the primers extended to detect or quantify the marker substances included in the sequences D01-D0n, thereby detecting or quantifying the target nucleic acids.

20 14. The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag(s) FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in
25 the order mentioned,

(i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule

is attached, and ED sequence, as primers;

(ii) binding a binding molecule bound to the SD sequence to a substance capable of being paired up therewith, thereby recovering a PCR product;

5 (iii) denaturing the PCR produce into a single strand

(iv) hybridizing the single strand with primers D11'-D1n' labeled;

(v) extending the primers;

10 (vi) denaturing the primers extended into single strands;

(vii) hybridizing extended single strands of the primers with sequences D01-D0n to detect or quantify marker substances included in that sequences D01-D0n, thereby detecting or quantifying the target nucleic acid.

15 15. The method according to claims 10 to 12, wherein the decode reaction comprises, where said flag FL is a double-stranded sequence and said 4 units consist of SD, D0, D1 and ED each having an arbitrary sequence, bound to each other sequentially in the order mentioned; and

(i) performing PCR for a single-stranded sequence encoded using SD sequence to which a binding molecule is attached and ED sequence, as primers;

25 (ii) binding the binding molecule bound to the SD sequence to a substance capable of being paired up

therewith, thereby recovering a PCR product;

(iii) denaturing the PCR product into a single strand;

(iv) mixing the sequences Dln' labeled and D0n' labeled, thereby hybridizing the single strand with the sequences Dln' and D0n';

(v) ligating the sequence Dln' with the sequence D0n';

(vi) denaturing the sequences ligated into a single-stranded sequence;

(vii) hybridizing sequences D01-D0n with the single-stranded sequence labeled with a marker substance, to detect or quantify the marker substance, thereby detecting or quantifying the target nucleic acid.

16. The method according to any one of claims 1 to 13, wherein said first partial sequence and said second partial sequence are positioned next to each other.